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Salmonella and Fecal Indicator Bacteria Survival in Soils Amended with Poultry Manure

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1 Introduction

Salmonellosis affects 1.2 million Americans annually (Scallan et al. 2011), with tens of millions of cases worldwide each year (WHO 2013). Infections can be caused by consumption of contaminated seafood, meat, eggs, dairy,

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juice, fresh produce, or water (Dale et al. 2010; FDA 2012). Quality control plans, mandatory testing of eggs, and hazard analysis at critical control points (HACCP) are used to reduce the spread of *Salmonella* in the Unites States (US). Despite these efforts, persistence of *Salmonella* (SALM) in the farm environment continues to be a concern for dairy, livestock and poultry operations, and growers of vegetables and leafy greens (CDC 2010; Jacobsen and Bech 2012; Berghaus et al. 2013). Additionally, the risk of transport of manure-derived bacteria to surface and groundwater resources is a concern worldwide (Unc and Goss 2004; Dale et al. 2010; WHO 2012; USEPA 2013).

Iowa is the leading producer of eggs in the US and is also a major producer of broiler chickens (UDSA-NASS 2014). Poultry manure (PM) from confinement operations is typically stockpiled and applied in the spring or fall to provide nutrients necessary for production of corn (*Zea mays L.*) (Moore Jr. 1998; Sharpley et al. 1997; Jn-Baptiste et al. 2013). Along with beneficial nutrients, PM commonly contains *Salmonella* (SALM) and other enteric pathogens, (Kraft et al. 1969; Rodriguez et al. 2006), which can be transported between farms and to water resources. In this region of the US, a majority of the soils are derived from sediments deposited during the Pleistocene glacial period (Brady 1984), and as much as 50% of the agricultural fields have had subsurface drainage tiles installed to artificially lower the water table in these poorly drained soils. While these tiles generally reduce the potential for transport of soil and other contaminants from agricultural fields via runoff (Skaggs et al. 1994), they have been shown to transport significant loads of leached contaminants, including bacteria, to surface waters (Bakhsh et al. 2005; Kjaer et al. 2007; Pappas et al. 2008; Hoang et al. 2013; Hruby et al. 2016).

Fecal coliforms, *Escherichia coli* (EC), and enterococci (ENT) are often used as indicators of the presence of fecal contamination in the environment. These fecal indicator bacteria (FIB) are preferred over direct detection of pathogenic organisms, because (1) they are present at high concentrations in human and animal waste, (2) they are generally commensal (not pathogenic), and (3) they are more easily, and less expensively, detected using culture methods. Epidemiological studies have shown associations between FIB and human illness after exposure to environmental waters (Pruss 1998). However, a growing body of literature suggests that FIB are not always well correlated to pathogen occurrence, and the fate and transport of these species can vary widely depending on their source and local environmental conditions (Payment and Locas 2011). In aquatic environments, SALM has been detected when FIB are absent or present at low concentrations (Morinigo et al. 1990; Polo et al. 1998, 1999).

Numerous laboratory studies have been conducted to assess the potential for survival of SALM, and other bacteria, in manure-amended soils. Key factors shown to impact bacterial survival include temperature, soil type, soil moisture, nutrient availability, and protection from UV exposure (Zibilske and Weaver 1978; Chandler and Craven 1980; Crane and Moore 1986; Guan and Holley 2003; Holley et al. 2006; You et al. 2006; Lang and Smith 2007; Garcia et al. 2010). Bacterial survival in soils is also dependent on interactions with plants and plant roots, protozoan predators, and native microbial communities (Jiang et al. 2002; Brandl et al. 2005; You et al. 2006; van Elsas et al. 2007; Garcia et al. 2010; Ibekwe et al. 2010; Liang et al. 2011; Rothrock et al. 2012; Farhangi et al. 2013; Erickson et al. 2014).

With such a wide range of complex interactions occurring in natural settings, it is especially important to collect field data from a variety of locations under a range of climate conditions. In addition, various common agricultural practices, such as storage practices, method of application, timing of application, and tillage practices, have been shown to impact bacterial survival and must be considered (Hutchison et al. 2004b; Arrus et al. 2006; Coelho et al. 2007; Semenov et al. 2009; Samarajeewa et al. 2012; Amin et al. 2013; Hoang et al. 2013). While numerous studies have documented persistence of SALM, and other pathogens in soils amended with swine and bovine manures, fewer have evaluated bacterial survival on artificially drained glacial till-derived soils (Gessel et al. 2004; Rogers et al. 2011; Samarajeewa et al. 2012; Hoang et al. 2013; Garder et al. 2014). Islam et al. (2004) observed longer survival times of SALM in soils amended with composted PM in comparison to composts from other manure sources; however, studies of untreated PM are rare. All previous field studies of bacterial survival in soils after PM application have been conducted in the Piedmont region of the southeastern US and have evaluated the effects of broiler litter application on cotton fields or grassed plots (Jangid et al. 2008; McLaughlin et al. 2011; Jenkins et al. 2012; Cook et al. 2014; Erickson et al. 2014). Of these studies, only one detected SALM in poultry litter samples (Cook et al. 2014).

The objective of our study was to evaluate the survival of SALM and FIB (EC and ENT) in Canisteo-Clarion-Nicollet series soils in cornfields amended with poultry

manure. Soil samples were collected each spring for 3 years (2010–2012) to evaluate if target bacteria persisted a full year after PM application, including over winter. Survival during the growing season was evaluated by measuring bacterial occurrence and concentrations in soils 35 days before, and 21, 42, and 158 days after manure application (DAM) in 2012, to determine the effects of time, tillage, and application rate. Finally, bacterial decay rates were estimated using records of target bacterial concentrations in drainage tile-waters collected during peak flows in a year with above-average precipitation (2010).

2 Materials and Methods

2.1 Study Site

Field experiments were conducted from 2010 through 2012 at Iowa State University's (ISU's) Agronomy and Agricultural Engineering Research Farm west of Ames, IA. The site is located in the Des Moines Lobe landform region, a landscape formed by the last glacial maximum that occurred in the Upper Midwest during the late Pleistocene Epoch, between 18,000–15,000 years ago. The research plots are located on soils with a Canisteo-Clarion-Nicollet association, which are loamy soils formed in glacial till under prairie vegetation, characterized as moderately permeable, with drainage classifications ranging from well-drained to poorly drained. Soil texture typically ranges from 30 to 45% sand, 35–42% silt, and 20–30% clay content (NRCS 2014). Topsoil (0–30 cm) measurements for all plots (2010–2012) range from 2.0 to 4.4% organic matter content. Plot slopes range from 0 to 5%. Tile drains are installed along the midline of each plot at a depth of approximately 1.2 m to enhance drainage. Plot areas range from 0.08 to 0.51 ha as described in Hruby et al. (2016). Tiles are spaced 36.3 m apart.

After 12 years of evaluation of the effects of PM application on split corn-soybean rotations (Nguyen et al. 2013), CP plots were converted to a continuous corn rotation for this study. The pattern of treatments followed the randomized study design established prior to 2010 on the CP plots, with three plots receiving lower rates of PM application (PM1), three plots receiving higher rates of application (PM2), and three plots receiving no manure as controls (PM0). PM1 and PM2 application rates were established based on the nitrogen (N) content of the manure, with the target rate for PM1 equivalent to 112 N kg ha^{-1}, and 224 kg N ha^{-1} for PM2, which is the maximum recommended rate of N application for continuous corn production in Iowa (Sawyer et al. 2006). Two of the control plots were fertilized with urea ammonium nitrate at 224 kg N ha^{-1} (PM0-UAN), and one that received no fertilizer (PM0-NONE). Poultry manure and UAN were applied to field plots by surface broadcast and incorporated into the soils by tilling to a depth of approximately 15 cm. Three adjacent NT plots were added to the study in 2010 and fertilized with PM1, PM2, and PM0-UAN treatments. No manure was applied to either type of PM0 plot; thus, we will not distinguish between PM0 treatments for the remainder of this article. Six possible combinations of tillage and treatment are as follows: CP PM0, CP PM1, CP PM2, NT PM0, NT PM1, and NT PM2.

2.2 Precipitation and Soil Temperature and Moisture Measurements

Rainfall data were collected using two tipping-bucket rain gauges with HOBO data-loggers (Onset Computer Corp., Pocasset, MA) located at the site. Daily average soil temperature data from 10.2-cm depth, and soil moisture percentage from a loam soil at 5-cm depth was obtained from the Soil Climate Analysis Network Site 2031 (NRCS 2014). Where continuous soil moisture data were missing, data from the Iowa Soil Erosion Project were used (IDEP 2014).

2.3 Manure Sampling, Application, and Analysis

Poultry manure from a confinement facility housing layer-hens (*Gallus gallus domesticus*) was transported to the study site, where it was stockpiled for up to 4 weeks, prior to application. Three representative PM samples were collected from the stockpile, placed in plastic bags and stored on ice, and then transported to Minnesota Valley Testing Laboratory in Nevada, IA, for nutrient analyses. Nitrogen content was used to calculate appropriate tonnage of manure to be applied per plot (Hanna and Richard 2008). Manure was resampled the day before application, and transported on ice to ISU's Water Quality Research Laboratory (WQRL) for moisture content and microbial analyses. Additional 8–12 PM samples were collected directly from the spreader during application in 2011 and 2012 for improved characterization of the microbial content. Application occurred on May 24 and 25, 2010, June 1, 2011, and May 15, 2012, followed immediately by tillage of CP

plots. Corn was planted within 3 days after PM application.

2.4 Soil Sampling

Soil samples (0–15- and 15–30-cm deep) were collected using hollow-core samplers (Oakfield Model L Tube Sampler with a 30-cm tube). Soil samples were collected prior to PM application in each year and on several dates following application in 2012. Samples were collected on May 17, 2010 (368 DAM), April 2, 2011 (374 DAM), April 10, 2012 (314 DAM in 2011 and 35 days prior to the 2012 application), July 5, 2012 (21 DAM), July 26, 2012 (42 DAM), and October 20, 2012 (158 DAM). No deep sample (15–30 cm) data are available for 2011 due to a laboratory processing error. For each plot, ten subsamples were obtained along diagonal transects, composited, and stored in plastic bags. Decontamination of all sampling equipment between plots was completed using a 90% ethanol solution. Samples were placed on ice and transported to the WQRL for moisture content and microbial analyses as described below.

2.5 Tile-Water Sampling

Water samples were collected directly from drainage tile outlets beginning after manure application and continuing for 100 DAM in 2010 as described in Hruby et al. (2016). Samples were collected in 1-L sterile polypropylene bottles, placed on ice, and transported to the WQRL, where they were stored at 4 °C until they were processed for EC and ENT within 24 h as described below.

2.6 Enumeration of *Salmonella* and FIB

Manure, soil, and water samples were analyzed using membrane filtration and growth on selective agars using previously described methods (Eaton et al. 1995; Messer and Dufour 1998; EPA 2002). Manure and soil subsamples (10 g wet weight) were mixed with 150 mL phosphate-buffered solution (PBS) and placed on an orbital shaker for 30 min to disperse aggregates and bioflocculated cells. Manure and soil solutions, and tile-water samples, were filtered in volumes ranging from 1 to 10 mL, including dilutions when necessary, to achieve bacteria plate counts ideally between 20 and 80 colonies. EC, ENT, and SALM were cultured on modified mTEC, mEnterococcus, and XLD agars, respectively (Difco™). Samples were analyzed in triplicate, and average values of replicates were used as the basis for further statistical analyses. For quality control, blanks with a minimum of 25 mL PBS were evaluated with each batch of samples. All bacteria concentrations are reported in colony-forming units (cfu) per gram on a dry weight basis for soil and manure and cfu/100 mL for water samples. Cultures of *E. coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Salmonella enteritidis* (ATCC 13076) were used as positive controls. Soil and PM samples were analyzed for moisture content following the ATSM D2216 standard procedure (ASTM 1998). Variations in soil sample volumes resulted in detection limits of 12, 19, and 14 cfu/g dry weight for 2010, 2011, and 2012 soil samples, respectively.

2.7 Data Analysis

Differences between distributions of bacterial application rates and bacterial concentrations in soil were determined by non-parametric Wilcoxon rank sum tests using JMP software (SAS Institute). Soil samples from 2012 were grouped by date relative to manure application (−35, 24, 42, and 158 DAM), treatment (PM0, PM1, and PM2), tillage (CP and NT), and sample depth (0–15 and 15–30 cm). For these analyses, all non-detections were assigned the value of the detection limit. Because differences between bacteria concentrations are not apparent when detection limits are low (< 33%), differences between frequency of positive detections were also assessed for tillage practices by Fisher exact tests using JMP software (SAS Institute).

Geometric mean bacterial concentrations in water during the top 10% of tile flow rates in response to precipitation events from field replicates were used for the following decay analysis, with the exception of the NT PM0 treatment, which was not replicated. In this analysis, it was assumed that target bacterial concentrations leached are proportional to the soil populations and the fraction transported remains constant throughout the season. Reductions in these concentrations over time were then used to estimate bacterial decay rates as described by Chick's law (described in Crane and Moore 1986):

$$N = N_0 e^{(-\mu t)} \quad (1)$$

where N is the bacterial concentration at time t (days), N_0 is the initial bacteria concentration, and μ is the die-

off rate constant (day^{-1}). Target bacteria were not detected in tile-water until 11 DAM, and the first peak flow occurred 19 DAM; therefore, t_0 was defined at 19 DAM. Decline of peak tile-water bacterial concentrations were fit with an exponential decay model (Eq. 1) in semi-log space and R^2 values, root mean square error (RMSE), and p values were determined by linear regression using JMP (SAS Institute). T_{90} values, the number of days for a 1-log reduction in tile-water concentrations of EC and ENT, are reported as determined from estimated decay curves.

3 Results and Discussion

3.1 Field Conditions

The average total rainfall for the field season (March to October) from 1998 to 2012 was 78 cm. In 2011 and 2012, rainfall was below-average, with 50 and 42 cm of precipitation, respectively. In contrast, 2010 was the wettest year at the field site since 1993, with 123 cm of rainfall. Soil moisture data from the nearby weather station reflect changes in precipitation until February 2012, after which, soil moisture data are not available. An alternative source of soil moisture data, the Iowa Daily Erosion project, reported an average volumetric soil moisture content at this location of 27–30% until early July (42 DAM), then average monthly soil moisture dropped to 15–20% for the remainder of the 2012 growing season (IDEP 2014). Soil moisture content of samples consistently averaged 15% for all sample dates. Average daily soil temperatures ranged from −5 to 33 °C from 2009 to 2012 (Iowa Environmental Mesonet 2014). Figure 1 shows the daily average 10.2 cm soil temperatures along with the dates of manure application, 5 cm soil moisture measurements, and timing and temperatures of soil samples.

3.2 Poultry Manure

Average moisture content of PM samples collected prior to application were 48, 61, and 30% in 2010, 2011, and 2012, respectively, and average total nitrogen content of PM ranged from 1.3 to 3.7%. Manure was applied to field plots at rates ranging from 5 to 13 Mg ha $year^{-1}$ for PM1 treatments, and from 10 to 40 Mg ha^{-1} $year^{-1}$ for PM2 treatments (Table 1).

Reported concentrations of target bacteria in PM vary widely between studies. For this study, mean concentrations of target bacteria in PM sampled at the time of application were highest for ENT each year (ranging from 5.0×10^4 to 1.5×10^5 cfu/g), followed by SALM (ranging from 2.0×10^2 to 1.4×10^5 cfu/g), and EC (ranging from 2.4×10^1 to 2.6×10^3 cfu/g). These EC and ENT concentrations are lower than previously reported for fresh PM, while SALM concentrations exceed previously reported values. Terzich et al. (2000) reported EC concentrations between 10^5 and 10^{10} cfu on a dry weight basis for fresh PM. ENT concentrations ranging from 10^5 to 10^8 cfu/g dry weight were reported by Graham et al. (2009). Jenkins et al. (2008) reported detection of EC and ENT in PM, but no SALM was detected using

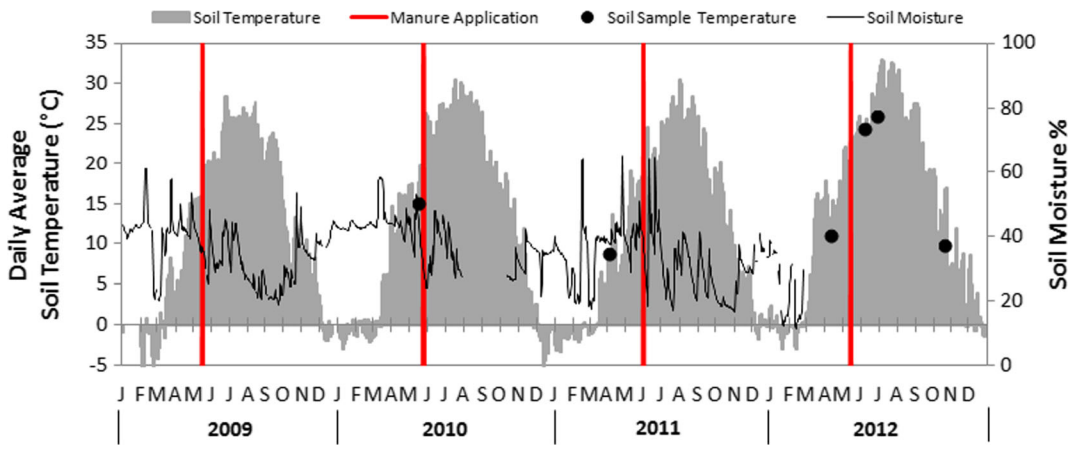

Fig. 1 Timing of soil samples and manure application with daily average 10.2 cm soil temperatures (°C) and daily average 5 cm soil moisture content (NRCS 2014)

Table 1 PM application rates, by total wet mass and plant available nitrogen

Year	Treatment	Tillage practice			
		CP		NT	
		Manure (Mg ha^{-1})	Nitrogen (kg N ha^{-1})	Manure (Mg ha^{-1})	Nitrogen (kg N ha^{-1})
2010	PM1	9.3 (2.4)[a]	72 (20)	12	90.2
	PM2	24 (0.1)	187 (1)	24	184
2011	PM1	13 (0.3)	120 (4)	12	109
	PM2	27 (1.6)	249 (25.8)	40	380
2012	PM1	6.0 (0.14)	132 (5.54)	5.0	103
	PM2	10 (0.15)	231 (5.96)	13	298

[a] Numbers in parentheses are standard deviations for rates on chisel-plowed plots

enrichment and culturing. McLaughlin et al. (2011) also did not detect SALM in PM using both culture and qPCR (using *spaQ* primers) methods. Cook et al. (2014) also did not detect SALM above their method detection limit of 100 cells/g without enrichment; however, they did confirm detection of SALM in over 50% of their PM samples using enrichment, followed by qPCR analyses for the *ttr* gene. Hutchison et al. (2004a) reported SALM concentrations in fresh PM samples up to 2.2×10^4 and 8.0×10^3 cfu/g for stored manure samples. Variations in bacterial concentrations of PM between studies could result from several variables, including the timing of PM collection, variations in on-farm management, and variations in bacterial shedding rates between flocks.

Target bacterial concentrations in PM did not correlate to N content of the manure, which was used to establish application rates, thus bacterial loading onto soils varied from year to year (Fig. 2). Wilcoxon rank sum comparisons indicate that bacterial application rates were significantly higher for PM2 than PM1 treatments within each year, with the exception of ENT in 2012, when no statistical differences between PM1 and PM2 treatments was observed.

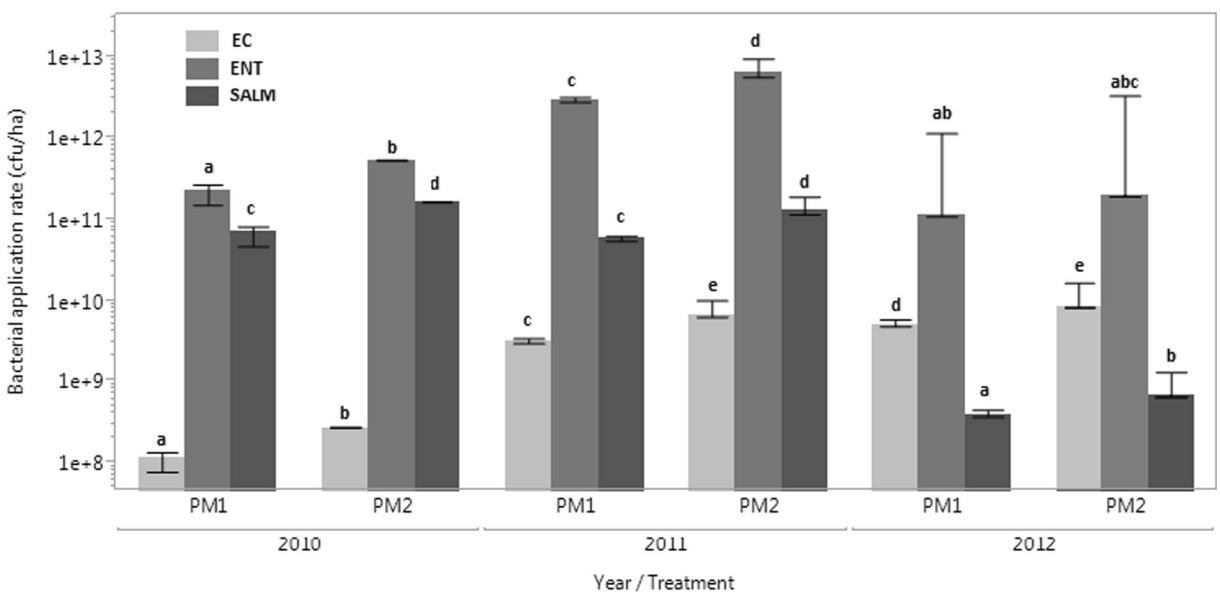

Fig. 2 Geometric mean estimated bacterial application rates on a per-hectare (ha) basis by treatment and year for PM amended plots. Bars indicate the range of bacterial application rates for each combination of year and treatment. Lettering indicates significant differences between application rates for each organism

3.3 Bacterial Survival in Soils

3.3.1 Long-Term Survival

The results of spring soil sampling suggest that populations of PM-derived bacteria can survive in soils almost a full year after PM application under some conditions. Target bacteria were not detected in soil samples in the spring of 2010, possibly due to lower inputs in 2009 when CP plots were split under a corn-soy rotation and only half of each plot received PM application (Nguyen et al. 2013), and NT plots received commercial fertilizer rather than manure. ENT and SALM were detected in 2011 and 2012 spring soil samples, approximately 1 year after application (Online Resource 1). These bacteria were not detected in samples from the control plot (PM0) in these years, suggesting that the history of PM application was responsible for the presence of these bacteria. EC were not detected in shallow soil samples from the spring of 2011 but were detected in both shallow and deep soil samples in the spring of 2012 in all PM2 plots, with a maximum concentration of 320 cfu/g. ENT were detected more frequently than either EC or SALM; however, ENT concentrations did not exceed 74 cfu/g. The maximum SALM concentration was 37 cfu/g. No discernible patterns were observed with regard to tillage or sample depth in spring soil samples.

Soil temperature and moisture conditions varied considerably between application of manure each spring and subsequent spring soil sampling (Fig. 1). Although multiple factors may be responsible for the lack of target bacteria in the spring of 2010, including lower application rates in 2009 (as described above), a contributing factor could have been the frequency of freeze-thaw cycles, which have been shown to decrease SALM survival (Olson et al. 1981). Eleven freeze-thaw cycles were observed during the winter of 2009–2010 compared to the winters of 2010–2011 (3) and 2011–2012 (5).

This is the first study to find differences between EC, ENT, and SALM concentrations in PM-amended and non-manured soils 1 year after PM application. McLaughlin et al. (2011) compared bacteria concentrations in soils with and without a history of PM (broiler manure) fertilization. These authors did not detect the pathogens, SALM or *Campylobacter* spp., or find differences between EC or ENT concentrations in manure-amended and non-manured fields, 1 year after PM application. The lack of differences between manured and non-manured plots in the McLaughlin et al. (2011) study may have been the result of lower application rates, soil types, or environmental variables. Application rates in the McLaughlin et al. (2011) study ranged from 2.2 to 13.4 Mg/ha per year, which are comparable to our PM1 treatment, and lower than our PM2 treatment.

3.3.2 Short-Term Survival in a Dry Year

Patterns of detection and occurrence in 2012 indicate that EC, ENT, and SALM respond differently to field conditions (Table 2). Concentrations of EC in pre-manure samples were higher than in any of the post-manure samples in 2012 indicating that any EC introduced by the manure survived less than 21 days. ENT were the most commonly detected species of target bacteria in 2012 soil samples, and statistically higher concentrations of ENT were seen at 21 and 158 DAM than in pre-manure samples. Concentrations of SALM were statistically higher 42 DAM than in pre-manure samples, but not in samples collected 21 and 158 DAM. Low detection frequencies of EC and SALM limited the statistical strength of these analyses. In addition, greater differences between pre- and post-manure samples may have been detectable if samples had been obtained less than 21 days after PM application. Gessel et al. (2004) found concentrations above background levels of *Salmonella* Anatum ($p = 0.0530$) in soils at 2-cm depths, 4 days after surface application of liquid swine manure and disking, but not in samples collected 7 or more DAM.

Analysis of post-manure application soil samples from 2012, reveal some effects of PM treatment, but again, low detection rates for EC and SALM limit statistical analyses. Wilcoxon rank sum tests reveal higher concentrations of ENT under plots that received the PM2 application rate, compared to the PM0 treatment ($p = 0.0067$), but no other significant differences were observed for post-manure soil bacteria concentrations (Table 3). Detection frequencies increase with increased PM application for EC and ENT, but not SALM (Table 3).

One explanation for the presence of ENT and SALM in control plots is that cross-contamination occurred between plots following PM application. This explanation is supported by increases in detection frequencies of ENT over time in control plots. ENT detection increased from 25% at 21 DAM to 38% at 42 DAM and reached 100% of samples 158 DAM (data not shown). SALM

Table 2 Summary statistics for *E. coli*, enterococci, and *Salmonella* spp. in soil samples from 35 days before, and 21, 42, and 158 days after manure application in 2012. The number of samples for each category is 16

Days after manure	Statistic	*E. coli*	Enterococci	*Salmonella*
− 35	Det. freq. (%)	44	56	25
	Median (cfu/g soil)	<14 b	< 14 a	< 14 a
	Max. (cfu/g soil)	840	40	23
+ 21	Det. freq. (%)	19	69	6
	Median (cfu/g soil)	< 14 a	< 14 bc	< 14 a
	Max. (cfu/g soil)	19	4500	< 14
+ 42	Det. freq. (%)	0	94	31
	Median (cfu/g soil)	< 14 a	27 ab	< 14 b
	Max. (cfu/g soil)	< 14	2600	790
+ 158	Det. freq. (%)	25	94	6
	Median (cfu/g soil)	< 14 a	200 c	< 14 a
	Max. (cfu/g soil)	< 14	56,000	190

Letters indicate statistically significant differences ($p < 0.05$) evaluated by pairwise comparison of pre- and post-manure sample sets using Wilcoxon rank sum analyses

detection in control plots was highest at 21 DAM (38%) and decreased to 13% at 42 and 158 DAM (data not shown). Despite all efforts to prevent cross-contamination, wind may have allowed some PM to reach control plots during broadcasting. The movement of wildlife, farm equipment, and subsurface waters could have facilitated contamination post-manure application. Overland flow was prevented by small berms between plots and standing water was never observed during 2012. CP plots were adjacent to one another but well separate from NT plots; therefore, cross-contamination between tillage practices was minimized.

The effects of tillage practices on soil bacteria detections and concentrations were evaluated for post-manure application samples in 2012 (Table 4). Because PM was incorporated shortly after application to CP plots, minimizing exposure of bacteria to ultraviolet light, we expected target bacteria to survive better in these plots than in NT plots where manure remained at the soil surface, as has been previously demonstrated (Tyrrel and Quinton 2003; Hutchison et al. 2004b; Rogers and Haines 2005). In fact, only SALM appeared to benefit from tillage, with 20% detection in CP post-manure soil samples, compared to 5% for NT samples (Table 4). Differences in detection frequencies and concentrations between CP and NT soils for SALM were statistically significant. Occurrence of EC and ENT were slightly higher in NT plots than CP, but the difference in detection frequencies was not significant (Table 4). Our results are consistent with previous studies of the impact of tillage on soil bacteria concentrations after PM application. Jenkins et al. (2008) found no differences between EC concentrations in soil samples from CP and NT plots 1 day after PM application and rainfall simulation. Cook et al. (2014) also found no effect of tillage on ENT concentrations in soils after PM application. Both Jenkins et al. (2008) and Cook et al. (2014) report more frequent SALM detection under CP plots than in NT soils.

Table 3 Summary statistics for EC, ENT, and SALM spp. in soil samples from control plots (PM0) and manure-treated plots (PM1 and PM2) after manure application in 2012. The number of samples for each category is 24

Treatment	Statistic	*E. coli*	Enterococci	*Salmonella*
PM0	Det. freq. (%)	0	46	21
	Median (cfu/g soil)	< 14 a	< 14 a	< 14 a
	Max. (cfu/g soil)	< 14	150	59
PM1	Det. freq. (%)	4	79	4
	Median (cfu/g soil)	< 14 a	27 ab	< 14 a
	Max. (cfu/g soil)	< 14	56,000	41
PM2	Det. freq. (%)	25	92	25
	Median (cfu/g soil)	< 14 a	87 b	< 14 a
	Max. (cfu/g soil)	19	6300	790

Lettering indicates differences between distributions of values in each category at $p < 0.05$

Table 4 Summary statistics for bacteria in post-manure application soil samples from 2012 by tillage class (CP = chisel plowed, NT = no-till)

Tillage	Statistic	*E. coli*	Enterococci	*Salmonella*
CP	*N*	54	54	54
	Det. freq.(%)	9 a	74 a	20 a
	Median (cfu/g soil)	14 a	37 a	14 a
	Max (cfu/g soil)	19	6300	790
NT	*N*	18	18	18
	Det. freq.(%)	11 a	78 a	5 b
	Median (cfu/g soil)	14 a	16 a	14 b
	Max (cfu/g soil)	14	56,000	14

Lettering indicate significant differences at $p < 0.05$ using Fisher exact test analyses for detection frequencies and Wicoxon rank sum analyses for comparisons between distributions of bacterial concentrations

These results highlight the difficulty of assessing effects of management practices on bacterial populations in soils where distribution of bacteria is likely to be highly heterogeneous as a result of manure broadcasting techniques. Extraction and homogenization of much larger soil samples may have improved our ability to identify differences resulting from varied tillage and application rates. However, PM is often clumped within samples and only very robust mixing techniques are likely to effectively disperse bacteria throughout the soil. Assessment of bacterial concentrations in drainage-tile waters may be a preferable method for evaluating bacterial survival, as described below.

3.4 Decay Rate Estimation Based on Peak Tile Water Concentrations

Figures 3 and 4 show the average peak concentrations of EC and ENT, associated tile flow rates, rainfall intensity, and the resulting estimated decay curves (Table 5). Post-manure EC and ENT concentrations in drainage-tile-waters remained above pre-manure values for 100

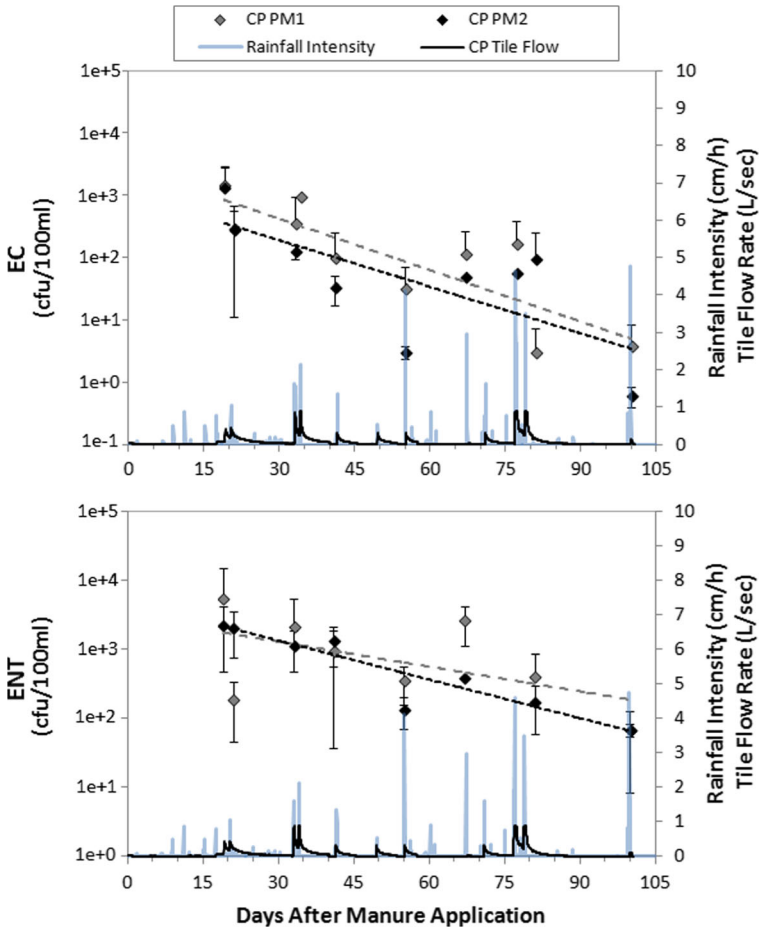

Fig. 3 Bacterial decay estimated from 2010 tile-water concentrations of EC and ENT after poultry manure application to CP plots. PM1 and PM2 represent low and high rates of poultry manure application. Error bars represent standard deviations of field replicates

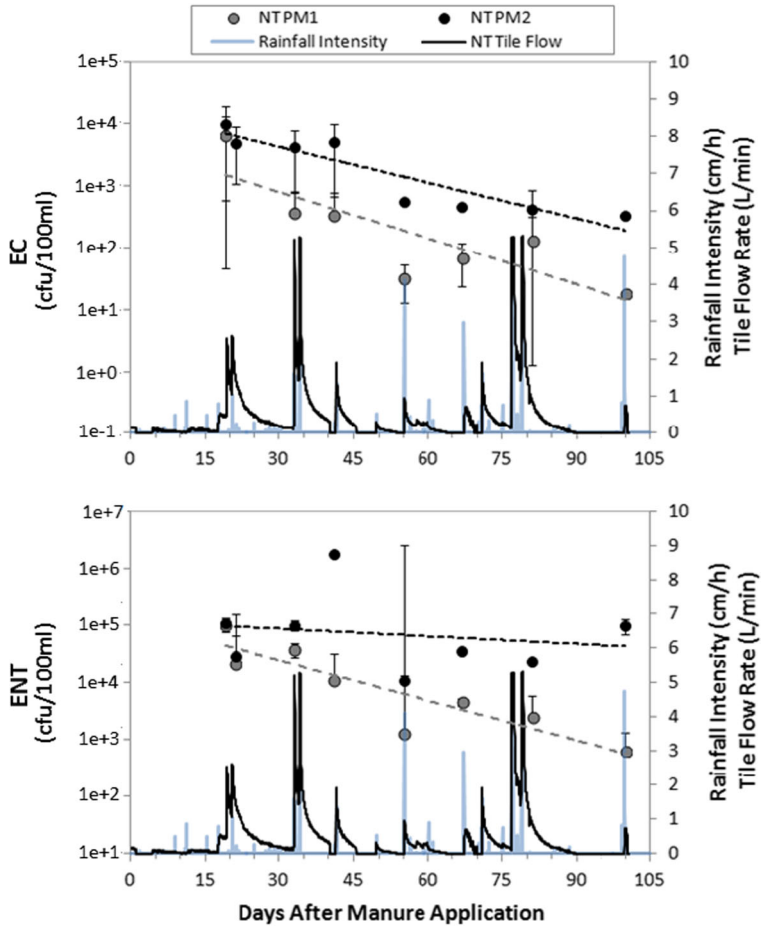

Fig. 4 Bacterial decay estimated from 2010 tile-water concentrations of EC and ENT after poultry manure application to NT plots. PM1 and PM2 represent low and high rates of poultry manure application. Error bars represent standard deviations of field replicates

DAM (Hruby et al. 2016). Peak tile-water bacterial concentrations measured during the top 10% of tile flow rates were used to estimate bacterial decay rates. Maximum tile-water bacteria concentrations were seen earlier (11 DAM) under NT plots than under CP plots (19 DAM). The tile flow rates in Figs. 3 and 4 are plotted in different units due to the order of magnitude differences in flow rates between chisel-plowed and no-till plots.

Estimated decay rates (Table 5) were higher for EC than ENT under all combinations of tillage and manure treatment. The lowest estimated decay constants for ENT under CP PM1 and NT PM2 treatments are suspect given their poor correlations. However, it is also possible that these values reflect regrowth of ENT under repeated wetting, as described by Yamahara et al. (2009) in marine beach sands. Decay estimates were derived from tile-water bacteria concentrations obtained 19–100 days after PM application; therefore, these rates potentially represent the second phase of decay as defined by (Benham et al. 2006). In controlled laboratory studies, Rogers et al. (2011) identified a second phase of decay beginning 10.7 to 16.9 days after inoculation in swine manure-amended soils for EC and 7.09 to 16.9 days for ENT in beef manure-amended soils (at 10 and 25 °C).

Although estimating decay from tile-water concentrations is an indirect method, it circumvents challenges in detecting low level concentrations in soil. Also, this method effectively integrates large volumes of soil, rather than relying on the low ratio of soil samples to potential contributing soil volumes in the field. The results of our estimation of decay appear reasonable with respect to previously published values under similar, but not identical, conditions. Estimated decay rates from 2010 are slightly higher than decay rates reported for the second phase of die off by Rogers et al. (2011) for soils amended with swine and beef manure in a laboratory setting. Rogers et al. (2011) reported decay

Table 5 Descriptive values for estimations of decay based on linear regressions of tile-water concentrations of EC and ENT under CP and NT plots with low (PM1) and high (PM2) rates of manure application in 2010

Bacteria	Tillage	Treatment	μ (day^{-1})[a]	T_{90}[b]	R^2	RMSE	*p*
EC	CP	PM1	0.065	35	0.55	217	0.0135
		PM2	0.063	37	0.90	73	0.0001
	NT	PM1	0.057	41	0.84	231	0.0036
		PM2	0.044	53	0.79	1217	0.0020
ENT	CP	PM1	0.028	83	0.27	556	0.1894
		PM2	0.043	54	0.93	218	0.0001
	NT	PM1	0.054	44	0.66	10,940	0.0144
		PM2	0.010	230	0.02	21,274	0.7619

RMSE root mean square error

[a] Decay constants (μ)

[b] Number of days for 1-log (or 90%) reduction in bacterial concentrations (T90)

constants ranging from 0.029–0.048 day^{-1} for EC and 0.011–0.030 day^{-1} for ENT, in soils incubated at 25 °C for 120 days. Soils in our study experienced a range of temperatures from 15 to 30 °C over the course of the 2010 growing season.

Decay constants were lower than those reported by Cook et al. (2014), who sampled 0–15 cm Crider silt-loam soils after poultry-litter application to grassed plots in 2011 and 2012. Cook et al. (2014) reported decay constants ranging from 0.17 to 0.31, corresponding to T_{90} values ranging from 7.41 to 13.31 days. Cook et al. (2014) used soil measurements from 1 to 148 days after application to estimate decay; therefore, their results are likely to represent the combined effects of the first- and second phases of decay as described by Benham et al. (2006), while our results are likely from the second phase.

While we were unable to determine a decay rate for SALM, the survival of SALM for over 80 days is consistent with the results of Rogers et al. (2011), who calculated a second-phase decay rate of 0.065–0.13 day^{-1} for *Salmonella enterica* serovar Typhimurium in swine manure-amended soils and 0.14–0.19 day^{-1} for beef manure-amended soils.

Tillage practices do not appear to impact EC or ENT decay rates estimated using 2010 tile-water bacteria concentrations. This is consistent with our analyses of 2012 soil data, which revealed no significant effects of tillage on EC and ENT. Cook et al. (2014) also reported no consistent differences between ENT decay rates in NT and CP soils amended with poultry litter (broilers) in their 2-year study.

4 Conclusions

Bacteria concentrations measured in Canisteo-Clarion-Nicollet silt-loam soil samples confirm that SALM and FIB can be detected up to 1 year after PM application, in the Midwestern USA. Although detection frequencies in 2012 soil samples were at or below 25% for EC and SALM, these target bacteria were detected 158 days after manure application despite drier than normal conditions. Both detection frequency and concentration of EC and ENT in soils increased with higher rates of PM application; however, the effect of application rate on SALM was inconclusive. Incorporation of SALM into soils via tillage favored persistence of SALM compared to surface application on NT soils. In contrast, tillage did not impact EC or ENT concentrations in soils, nor were differences observed in EC and ENT decay rates estimated from tile-water bacterial analyses. ENT detection frequencies and concentrations were consistently greater than both SALM and EC in manure, soil, and water samples. Previous studies suggest that ENT could potentially be used as an indicator of risk from PM-derived SALM. However, detection of ENT in control plots (PM0) suggests that background levels of ENT should be established before inferring the presence of pathogens. Overall, our results indicate that soils act as a long-term reservoir for manure-derived *Salmonella*, and that the potential for release of these pathogens to the environment should be considered when making manure management decisions.

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